

Comparison of anti-inflammatory effects between UV and ionizing radiation in co-cultures of macrophages and lymphocytes*

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Introduction

Chronic inflammatory diseases such as arthritic disorders are efficiently treated by irradiation with low doses of photons or α -particles [1,2]. However, the molecular and cellular background of the anti-inflammatory effects by low dose irradiation remains widely unknown. It was shown that the phagocytosis of UV induced apoptotic cells has an immunosuppressive effect on human macrophages, monocytes or leukocytes [3,4,5]. Up to now, the immune-suppressive effects of ionizing radiation have not been investigated yet. In the present work the question was raised if the induction of apoptosis through ionizing radiation would have the same effect on human macrophages as described for UV irradiation. To achieve this goal a simple phagocytosis assay of mature macrophages and apoptotic lymphocytes was established for a quantitative measurement of cytokine release.

Materials and Methods

Mature macrophages were generated by *in vitro* differentiation for 7d with M-CSF [50ng/ml]. For the phagocytosis assay human peripheral blood lymphocytes (PBL) were irradiated using X-rays (250kV, 16mA) or UV-B. After irradiation PBL and macrophages were co-cultured for 20h. For all experiments macrophages were cultured alone as controls for the cytokine release of the monocultures. After co-incubation the supernatant was removed, centrifuged and subsequently stored at -80°C. The remaining unbound PBL were removed by washing with PBS, afterwards macrophages were detached and the cell number was determined. The concentration of the cytokines: TGF- β and IL-10 (anti-inflammatory) and TNF- α (pro-inflammatory) were measured with ELISA technique (eBiosciences), according to the manufacture instructions.

Results and Conclusion

The results for the release of TGF- β , IL-10 and TNF- α of the co-cultures are shown in figure 1. For a better comparison between unirradiated and irradiated samples, the released amount of cytokines was normalized on the cell number of each sample. The results are shown as relative cytokine release, in which the release of the monocultures of macrophages was set as 1. The concentrations of the three cytokines were ranging from: 130-220pg (TGF- β), 24-220pg (IL-10) and 2-4pg (TNF- α). As can be seen from figure 1 only minor changes could be detected for the release of TGF- β and TNF- α . However the release of IL-10 was remarkably increased (6 fold) after co-incubation of macrophages with apoptotic PBL which

were irradiated with UV-B (180mJ/cm²), while irradiation with X-rays (6Gy) had no effect on the IL-10 release.

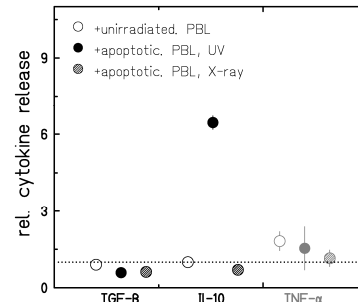


Figure 1: Relative cytokine release of human macrophages, co-cultivated with unirradiated- or apoptotic PBL. Apoptosis was induced by irradiation with X-ray (6Gy) or UV-B (180mJ/cm²). Co-incubation was performed for 20h. Error bars are shown as SEM. (N=2, n=4)

The observed increased release of the anti-inflammatory cytokine IL-10 by macrophages after co-incubation with PBL, that were apoptotic after UV-B exposure is in good agreement to published data [4]. However this effect could not be observed after irradiation with X-rays, where overall no modification of the cytokine release related to immune-suppressive effects was observed. Taken together, our results clearly show differences between X-rays and UV-B in their immunosuppressive effect.

A possible reason for the huge differences of the IL-10 release might be a different amount of induced late apoptosis or necrosis of the PBL. The relatively high UV-B irradiation caused a much higher induction of late apoptosis and necrosis in PBL compared to X-ray irradiation. For UV-B irradiation after 24h almost 90% of the cells were late apoptotic or necrotic, whereas for X-ray irradiation only 40% were apoptotic, without any necrotic cells. The higher amount of apoptotic or necrotic cells following UV exposure at the investigated time point may lead to an increased phagocytotic activity of the macrophages which in turn could influence the cytokine release. These questions will be assessed in future experiments.

References

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